An Examination of the Influence of Diet on the Growth, Development and Survival of *Manduca sexta*

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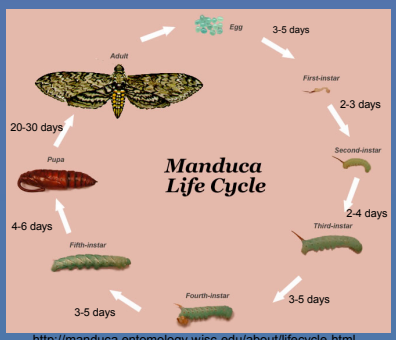
Introduction

In the wild, the tobacco hornworm, *Manduca sexta*, can be found on a variety of solanaceous plants including varieties of both tomato and tobacco species. Relatively few insects are solanaceous feeders because of toxic alkaloids present in the tissues of these plants. *Manduca sexta* however is able to sequester and secrete such toxins rendering them useless as a defense mechanism for the plant. *Manduca* are then able to consume large quantities of these plants with no adverse effects. It has long been established in the scientific community that *Manduca* is a specialist feeder, feeding only on members of the *Solanaceae* family. Recently however, researchers in the southwestern U.S. discovered previously unreported, non-*Solanaceae* species being routinely used as host plants for oviposition by *Manduca* females. Successful development and emergence from larvae to adult was reported, challenging what is known about this species (Mechaber and Hildebrand 2000). In laboratory reared Manduca, there is evidence to suggest that specificity for a given host plant is not inherited but rather is influenced by the act of feeding on that plant during the early stages of larval development. Thus larvae reared on a non-host plant from the early instar stages of development feed off such plants not just in cases of extreme starvation but as a normal diet. Likewise, larvae raised on an artificial diet were much more accepting of a non-host plant compared to those raised on a traditional solanaceous plant (Flowers and Yamimoto 1982). In this multi-week lab, students are asked to design and implement an experiment to investigate how nutrition influences the growth, development and survival of *Manduca sexta*.

The tobacco hornworm lifecycle



http://manduca.entomology.wisc.edu/about/lifecycle.html

Designing the experiment

When designing their experiment, students are asked to consider the following questions:

1) What type of diet contains the optimal nutrients for growth and development? Not all diets are toxic but this does not mean that all diets are created equal. One design choice could be to test several species of non-toxic plant to look at overall nutritional value.

2) Does toxicity level and the potential energy needed for sequestration affect growth rates? Toxins can cause death or merely delay growth depending on the ability of the organism to overcome a particular toxin. One design choice could be to compare a solanaceous plant with a non solanaceous toxic plant to look at how different types of toxins impact manduca growth.

3) What stage(s) of development are impacted most by diet?

Students need to consider that toxins can potentially impact early instars differently from later instars. One design choice could be to introduce a toxic diet in at different instars.

Diet preparation

The diet is a modification of that of Bell and Joachim (1976) and can be found at http://www.entm.purdue.edu/entomology/outreach/recipe/manduca.diet.htm Wheat germ is used as a base for this standard laboratory diet. Dried plant material can be ground up with a mortar and pestle and substituted for the wheat germ to create different diets.

Animal care and maintenance

Tobacco hornworms can be obtained as eggs from Carolina Biological Supply Company and maintained at room temperature in the classroom. Caterpillars can be housed in petri dishes until approximately the 4th instar at which point they should be moved into a larger container such as a paper soup cup. Food should be given in excess and the containers should be checked and cleaned if necessary every other day.

Data collection

Students should weigh and make note of the developmental stage of each caterpillar every other day.

Timeline

This lab is designed as a multi week experiment with the initial set up taking place during the students' weekly lab period. Group members are then responsible for checking on their experiment (approx. 10-15 minutes) every other day until pupation.

Week 1(optional):
Brine shrimp bioassay to determine potential toxicity in plants around campus (see handout). Alternatively, students could do literature research to determine toxicity.

Week 2:
Discuss results of brine shrimp assay, design experiment, collect plant material for drying

Week 3:
Make diets (alternatively this could be done by the instructor or students outside of lab during week 2), set up experiment

Week 4 -6:
Continue to feed Manduca and collect weight and instar data
Optional week 4 or 5: mini food preference experiment (see handout)

Results

The results of this project are as variable as the plants that the students decide to chose. Typical results range from delayed development (all catepillars in the picture to the right are the same age) to death depending on the toxicity and nutritional value of the plant chosen. When discussing the data, students need to consider that both toxicity and a lack of adequate nutrients can cause similar results so they will need to research their plant species as they are writing their papers.

Plants that my students have chosen in the past

Tobacco	Coleus
Tomato	Common periwinkle
Radish	English ivy
Oats	Grass
Rye	Spinach

References

•Bell, R.A. and F.G. Joachim. 1976. Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. Annuals of the Entomological Society of America 69(2):365-73
•Flowers, R.W. and R.T. Yamamoto. 1982. Feeding on non-host plants by the tobacco hornworm (Manduca sexta, Lepidopteridae). The Florida Entomologist 65(4):523-530
•Mechaber, W.L. and J.G. Hildebrand. 2000. Novel, non-solanaceous hostplant recorded for Manduca sexta (Lepidoptera:Sphingidae) in southwestern United States. Annuals of the Entomological Society of America 93(3):447-51.